RESEARCH ACTIVITIES IX
Center for Integrative Bioscience

IX-A Single-Molecule Physiology

A single molecule of protein (or RNA) enzyme acts as a machine which carries out a unique function in cellular activities. To elucidate the mechanisms of various molecular machines, we need to observe closely the behavior of individual molecules, because these machines, unlike man-made machines, operate stochastically and thus cannot be synchronized with each other. By attaching a tag that is huge compared to the size of a molecular machine, or a small tag such as a single fluorophore, we have been able to image the individual behaviors in real time under an optical microscope. Stepping rotation of the central subunit in a single molecule of F_1-ATPase has been videotaped, and now we can discuss its detailed mechanism. RNA polymerase has been shown to be a helical motor that rotates DNA during transcription. Myosin V is another helical motor that moves as a left-handed spiral on the right-handed actin helix. Single-molecule physiology is an emerging field of science in which one closely watches individual, "live" protein/RNA machines at work and examines their responses to external perturbations such as pulling and twisting. I personally believe that molecular machines operate by changing their conformations. Thus, detection of the conformational changes during function is our prime goal. Complementary use of huge and small tags is our major strategy towards this end.

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IX-A-1 The ATP-Waiting Conformation of Rotating F_1-ATPase Revealed by Single-Pair Fluorescence Resonance Energy Transfer

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F_1-ATPase is an ATP-driven rotary motor in which a rod-shaped γ subunit rotates inside a cylinder made of $\alpha_3\beta_3$ subunits. To elucidate the conformations of rotating F_1, we measured fluorescence resonance energy transfer (FRET) between a donor on one of the three βs and an acceptor on γ in single F_1 molecules. The yield of FRET changed stepwise at low ATP concentrations, reflecting the stepwise rotation of γ. In the ATP-waiting state, the FRET yields indicated a γ position ≈ 40° counterclockwise (= direction of rotation) from that in the crystal structures of mitochondrial F_1, suggesting that the crystal structures mimic a metastable state before product release.

IX-A-2 Single Molecule Imaging of the Rotation of F_1-ATPase

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A single molecule of F_1-ATPase has been shown to be a rotary motor, driven by adenosine triphosphate (ATP) hydrolysis, in which the central γ subunit rotates against a surrounding cylinder made of alternately arranged three α and three β subunits. Together with another (yet putative) proton-driven rotary motor F_o, it constitutes the F_oF_1-ATP synthase that synthesizes ATP from adenosine diphosphate (ADP) and inorganic phosphate using proton flow as the energy source. Isolated F_1 composed of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ subunits only hydrolyzes ATP, and hence is called F_1-ATPase. Its subcomplex $\alpha_3\beta_3\gamma$ suffices for rotation driven by ATP hydrolysis. Single-molecule imaging of this subcomplex has revealed detailed mechanical and kinetic properties of the motor1 activity, and high-resolution atomic structures of F_1 are already available. At present, F_1-ATPase is one of the best characterized molecular motors, or nucleotide-driven molecular machines. It is possible to learn a lot from this rotary machine about the molecular mechanism of chemo-mechanical energy transduction.

Because all molecular machines work stochastically, their operations can never be synchronized with each other in a rigorous sense. Thus, it is necessary to watch the individual behaviors closely. With F_1-ATPase, for example, we have been able to show that it rotates in a unique direction, that it does so in discrete 120° steps, and that 120° steps are resolved into ~ 90° and ~ 30° substeps at low ATP concentrations. We have also been able to measure its rotary torque, and have shown that its energy conversion efficiency can reach ~ 100%. We believe that it would be very difficult, if not impossible, to obtain these results without dealing with individual molecules. Here we describe in detail the techniques involved, hoping that they may also be applicable to other molecular machines, in particular to the detection of conformational changes underlying their function (note that a conformational change accompanies reorientation, or partial rotation, of one part against the other).

For the detection of rotation (or conformational changes), we recommend the complementary use of large and small probes. Here we describe two examples, an actin filament as a probe that is large compared to the rotary motor, and a single fluorophore as a small and

less perturbing probe. We begin with the preparation of materials, and proceed to the setting of functional motor molecules on a glass surface and then to imaging and analysis.